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Dose effects of propofol on hemodynamic and cytokine responses to endotoxemia in rats

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Abstract
Purpose. Our previous studies have demonstrated that propofol inhibits hypotension, metabolic acidosis, and cytokine responses and reduces mortality in endotoxemic rats. The purpose of this study was to elucidate whether these beneficial effects of propofol on hemodynamics and cytokine responses were dose related.
Methods. Forty-eight rats were divided at random among four equal groups: groups S, M, and L received intravenous propofol administration (5, 10, and 20 mg·kg^{-1}·h^{-1}, respectively) immediately after endotoxin (*Escherichia coli* endotoxin; 15 mg·kg^{-1}, i.v.) was given. Group E received endotoxin alone. We assessed hemodynamics and plasma cytokine [tumor necrosis factor (TNF)-α and interleukin (IL)-6] concentrations for 5 h following endotoxin injection.
Results. Systolic arterial pressure (SAP) was significantly higher at 4 and 5 h in groups S and M than in group E ($P < 0.05$), although SAP decreased progressively in all groups. Endotoxin injection increased the TNF-α and IL-6 concentrations in all groups. The increase in TNF-α concentrations at 2 h was significantly lower in group M than in group E ($P < 0.05$). On the other hand, the increase in IL-6 concentration at 5 h was significantly lower in groups M and L than in group E ($P < 0.05$).
Conclusion. The effects of propofol on blood pressure and cytokine responses were influenced by the dose of propofol, although the relationship did not follow simple linearity.

Key words Propofol · Endotoxemia · Cytokine response · Cardiovascular response

Introduction

Endotoxemia is a common predisposing factor to refractory endotoxin shock, characterized by profound hypotension, progressive metabolic acidosis, and multiple organ failure [1]. Circulating endotoxin increases production and release of tumor necrosis factor (TNF)-α and interleukin (IL)-6 [2–5]. Not only endotoxin but also cytokines have been implicated as important factors in the pathophysiology of endotoxemia [2–6]. Our previous study demonstrated that propofol administration inhibited metabolic acidosis, cytokine responses, and activation of neutrophils in endotoxemic rats [7]. We also documented that treatment with propofol after endotoxin injection drastically reduced mortality in rats [8]. However, we did not determine whether these beneficial effects of propofol were dose dependent. This study was undertaken to clarify the relationship between the dose of propofol and the magnitude of hemodynamic and cytokine responses to endotoxemia in rats.

Materials and methods

Animal preparation

Forty-eight male Wistar rats, weighing 352 ± 20 g (mean ± SD), were used in this study. All experimental procedures were approved by the Animal Care Committee of Kanazawa University, and were in accordance with the National Institute of Health guidelines for animal use. The method of animal preparation was reported previously [7]. Briefly, after an intraperitoneal injection of pentobarbital sodium (30 mg·kg^{-1}), ventilation was performed through a tracheotomy. The femoral artery and vein were cannulated, and lactated Ringer's solution containing pancuronium bromide (0.02 mg·ml^{-1}) and pentobarbital sodium (0.5 mg·ml^{-1}) was infused continuously (10 ml·kg^{-1}·h^{-1}) throughout the experiment. The rats were connected to a pressure-controlled ventilator (Servo 900C; Siemens-Elema, Solna, Sweden), which delivered 100% oxygen at a frequency of 30

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breaths/min with an inspiratory : expiratory ratio of 1 : 1. After these preparatory procedures, the animals were rested for more than 30 min to allow the blood gases and hemodynamic parameters to stabilize.

Experimental protocols

After baseline measurements, animals were allocated randomly to one of four groups ($n = 12$ per group).

Endotoxin-alone group (group E)
Endotoxin (15 $mg \cdot kg^{-1}$) was injected intravenously over 2 min to induce endotoxemia. We used lipopolysaccharide derived from *Escherichia coli* (0111:B4; Difco, Detroit, MI, USA) as endotoxin.

Small-dose treatment group (group S)
Propofol (1% propofol injection "Maruishi"; Maruishi Seiyaku, Osaka, Japan) was administered intravenously at a constant rate of 5 $mg \cdot kg^{-1} \cdot h^{-1}$, starting immediately after endotoxemia was induced as for group E. Propofol used in this study does not contain edetate disodium as a preservative.

Medium-dose treatment group (group M)
Propofol was administered at a constant rate of 10 $mg \cdot kg^{-1} \cdot h^{-1}$. Otherwise, the animals were handled identically as in group S.

Large-dose treatment group (group L)
Propofol was administered at a constant rate of 20 $mg \cdot kg^{-1} \cdot h^{-1}$. Otherwise, the animals were handled identically as in group S.

Rectal temperature was maintained between 36° and 38°C using a heating pad. An arterial blood sample (0.25 ml) was drawn hourly throughout the 5-h observation period for the measurement of arterial pH (pH_a), CO_2 tension (Pa_{CO_2}), O_2 tension (Pa_{O_2}), and the bicarbonate concentration. Furthermore, arterial blood samples (1.0 ml) were drawn for the measurement of plasma cytokine (TNF-α and IL-6) concentrations at 2, 4, and 5 h after the endotoxin injection. The total amount of blood drawn from each animal was 5.5 ml over 5 h.

Sample analysis

Blood samples drawn to determine cytokine concentrations were centrifuged for 10 min at 3000 *g* at 4°C. Plasma was then decanted and stored at −70°C until analysis. Cytokine concentrations were measured with an enzyme-linked immunosorbent assay (BioSource, Camarillo, CA, USA). The lower limits of detection for TNF-α and IL-6 were 4.5 $pg \cdot ml^{-1}$ and 7.0 $pg \cdot ml^{-1}$, respectively.

Statistical analysis

Data are presented as mean ± SD. Differences between groups at baseline were analyzed with unpaired Student's *t* test. Hemodynamic and cytokine changes during the study were analyzed by using two-way analysis of variance with repeated measures followed by a post hoc test (Bonferroni's method). Statistical analyses were performed using Stat View (version 5.0, Abacus Concepts, Berkeley, CA, USA). Statistical significance was defined as $P < 0.05$.

Results

Hemodynamics

No significant differences were noted in the systolic arterial pressure (SAP) at baseline among the four groups (Fig. 1). Endotoxin injection decreased SAP progressively, and SAP became significantly lower at 5 h after endotoxin injection compared with baseline values in all groups ($P < 0.05$). However, significantly higher SAP was observed at 4 and 5 h after endotoxin injection in groups S and M than in group E ($P < 0.05$), but not in group L ($P = 0.16$). Heart rate (HR) did not differ significantly among the four group at any point during the 5-h observation period (see Fig. 1).

Plasma cytokine concentrations

All baseline values of plasma cytokine concentrations did not differ significantly among the four groups (Fig. 2). Although endotoxin injection increased TNF-α concentration at 2 h after the injection in all groups, the concentration was significantly lower in group M ($P < 0.05$) than in group E, but not in groups S ($P = 0.18$) and L ($P = 0.32$). IL-6 concentration was increased progressively in all groups, but significantly lower IL-6 concentrations were observed at 5 h after endotoxin injection in groups M ($P < 0.05$) and L ($P < 0.05$) than in group E, but not in group S ($P = 0.96$). No significant differences between group M and group L were noted in the IL-6 concentration.

Blood gases

The Pa_{CO_2} and Pa_{O_2} values did not differ significantly among the four groups at any point during the 5-h observation period (Table 1). No significant differences were noted in baseline values of pH_a and base excess among four groups (Table 1). However, the pH_a and base excess values declined progressively in group E and were significantly lower at 5 h after endotoxin injection in group E than in all groups with propofol administration ($P < 0.05$).

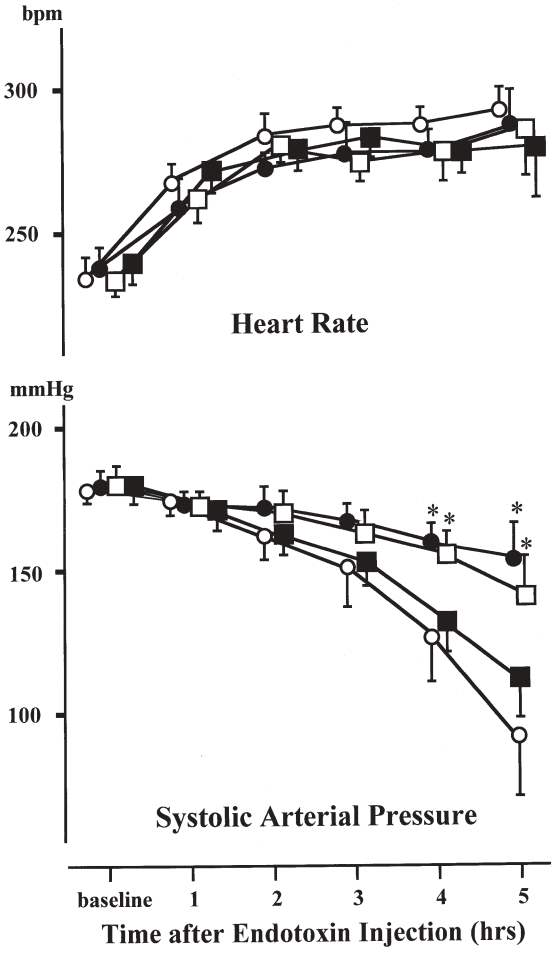

Fig. 1. Heart rate (*HR*) (*top*) and systolic arterial pressure (*SAP*) (*bottom*) at baseline and after endotoxin injection (mean ± SD). *Open circles*, endotoxin-alone group; *closed circles*, small-dose treatment group; *open squares*, medium-dose treatment group; *closed squares*, large-dose treatment group. *$P < 0.05$ vs endotoxin-alone group at each observation point

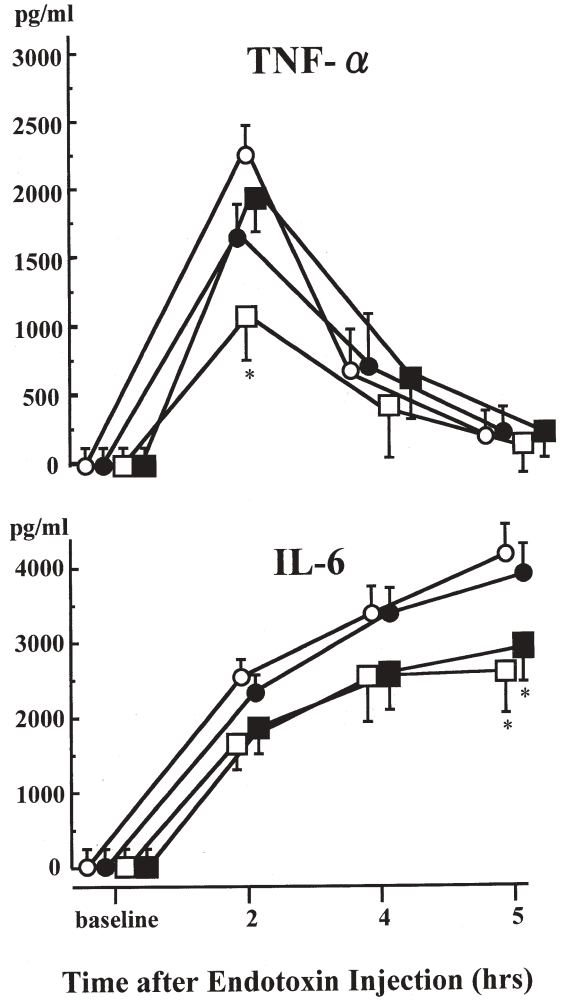

Fig. 2. Changes of plasma tumor necrosis factor (*TNF*)-α (*top*) and interleukin (*IL*)-6 (*bottom*) at baseline and after endotoxin injection (mean ± SD). *Open circles*, endotoxin-alone group; *closed circles*, small-dose treatment group; *open squares*, medium-dose treatment group; *closed squares*, large-dose treatment group. *$P < 0.05$ vs endotoxin-alone group at each observation point

Discussion

The results from groups E and M reconfirmed the results of our previous study that propofol inhibited hypotension, metabolic acidosis, and cytokine responses in rats injected with endotoxin [7,8]. The present study demonstrated that these effects of propofol were influenced by the dose of propofol. Small and medium doses of propofol attenuated the severity of hypotension in endotoxemic rats, but a large dose of propofol did not. Regarding the antiinflammatory effects, only a medium dose of propofol inhibited an increase in concentrations of both TNF-α and IL-6. These correlations among propofol dose and hemodynamic and cytokine responses to endotoxemia are the most important findings in our study.

Of particular interest is the relationship of propofol dose and antiinflammatory effects. Previous in vitro studies demonstrated conflicting results as to whether propofol exerts dose-related effects on endotoxemia [9,10], and in vivo studies are few. Our present study showed that only a medium dose of propofol inhibited an increase in the TNF-α concentration. In contrast, the inhibitory effects of propofol on IL-6 concentrations hit the ceiling in a large dose, and the antiinflammatory effects were demonstrated in a large dose as well as in a medium dose. Consequently, we have concluded that the effects of propofol on the concentrations of both TNF-α and IL-6 are dose independent.

Several investigations have indicated that a large dose of propofol administration reduced myocardial

Table 1. Arterial blood gas values at baseline and after endotoxin injection

	Time after injection (h)					
	Baseline	1	2	3	4	5
pH_a						
Endotoxin-alone group	7.47 ± 0.04	7.35 ± 0.04	7.36 ± 0.07	7.37 ± 0.07	7.36 ± 0.05	7.27 ± 0.06
Small-dose treatment group	7.47 ± 0.08	7.38 ± 0.05	7.40 ± 0.05	7.37 ± 0.06	7.40 ± 0.04	7.38 ± 0.05*
Medium-dose treatment group	7.47 ± 0.04	7.36 ± 0.06	7.39 ± 0.06	7.36 ± 0.05	7.41 ± 0.06	7.46 ± 0.09*
Large-dose treatment group	7.47 ± 0.04	7.39 ± 0.06	7.41 ± 0.06	7.41 ± 0.05	7.43 ± 0.07	7.41 ± 0.07*
Pa_{CO_2}(torr)						
Endotoxin-alone group	34 ± 6	38 ± 6	38 ± 4	35 ± 7	31 ± 4	33 ± 5
Small-dose treatment group	34 ± 6	36 ± 5	35 ± 3	36 ± 6	33 ± 6	34 ± 4
Medium-dose treatment group	35 ± 5	39 ± 5	36 ± 5	31 ± 6	37 ± 6	32 ± 6
Large-dose treatment group	34 ± 4	33 ± 6	34 ± 7	33 ± 6	33 ± 3	35 ± 5
Pa_{O_2}(torr)						
Endotoxin-alone group	557 ± 87	512 ± 37	551 ± 30	550 ± 48	548 ± 36	499 ± 67
Small-dose treatment group	545 ± 47	504 ± 43	495 ± 34	464 ± 61	486 ± 54	534 ± 48
Medium-dose treatment group	554 ± 43	493 ± 33	499 ± 56	455 ± 78	470 ± 71	483 ± 63
Large-dose treatment group	558 ± 55	528 ± 34	524 ± 22	511 ± 38	520 ± 59	553 ± 33
Base excess						
Endotoxin-alone group	1.3 ± 2.0	−4.0 ± 1.8	−4.5 ± 2.0	−4.7 ± 2.2	−7.2 ± 1.8	−9.5 ± 4.5
Small-dose treatment group	1.5 ± 3.0	−3.7 ± 2.0	−4.0 ± 2.3	−4.7 ± 2.6	−3.2 ± 2.1*	−3.8 ± 3.0*
Medium-dose treatment group	2.2 ± 1.5	−4.3 ± 1.6	−4.6 ± 2.5	−3.2 ± 2.4	−0.9 ± 1.3*	−0.8 ± 2.9*
Large-dose treatment group	1.5 ± 2.3	−3.2 ± 1.3	−3.5 ± 1.4	−3.4 ± 1.5	−1.3 ± 3.2*	−1.9 ± 2.3*

All data are mean ± SD
pH_a, arterial pH
* $P < 0.05$ vs endotoxine-alone group at each observation point

contractility and dilated peripheral vessels [11–13]. Our study showed that a large dose of propofol neither improved endotoxin-induced hypotension nor attenuated proinflammatory cytokine responses as much as a medium dose. These findings suggest that the antiinflammatory effects of propofol could be less prominent than the inhibition of cardiac function, and may be one of the reasons that the antiinflammatory effects of propofol were not dose dependent in vivo. Further investigations are needed to clarify the mechanisms responsible for the changes of antiinflammatory effects by the dosage of propofol.

An important question of whether lipid as a solvent for propofol has antiinflammatory effects remains unanswered. Heine et al. [14] documented that lipid inhibited the respiratory burst of neutrophils, whereas Mikawa et al. [9] showed that the amount of lipid contained in a propofol formulation had no effect on the reactive oxygen species generated by neutrophils. Further studies are needed to answer this question.

Critically ill patients with sepsis and in septic shock suffer a high degree of stress because of pain, anxiety, and organ-specific responses to sepsis. An important objective in the management of these patients is to achieve an adequate level of sedation. Our findings suggest that propofol may have the advantage of preventing inflammatory responses in septic patients, but this effect changes with propofol dose, and a large dose of propofol may even aggravate hypotension.

In summary, we reconfirmed the results of our previous study that administration of propofol antagonized hypotension, metabolic acidosis, and cytokine responses in endotoxemic rats. Moreover, the hemodynamic benefits and antiinflammatory effects of propofol were dose independent and were demonstrated by administration with an appropriate dose of propofol for endotoxemia. Although the mechanisms responsible for the beneficial effects require further investigation, our results suggest that judicious use of propofol as an anesthetic and sedative agent may have beneficial effects on endotoxemia.

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